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**Tumor-Derived Microvesicles and the Cancer Microenvironment**

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**Running title:** Microvesicles and cancer

**Abstract**

Tumor cells release microvesicles (MVs) that may remain in the extracellular space in proximity to the cell of origin, or that may migrate to distant sites by entering biological fluids. Increasing evidence indicates that MVs are mediators of cell-to-cell communication which are able to deliver specific signals, both within the tumor microenvironment and in the long-range. MVs are able to transfer bioactive lipids and proteins, including oncogene products and receptors, from the cell of origin to recipient cell. In addition, MVs may induce epigenetic changes in recipient cells by transferring genetic information in the form of mRNA, microRNA and oncogenes. Several changes in the phenotype and function that occur in stromal cells within the cancer microenvironment have been ascribed to tumor cell-derived MVs. In this review we discuss the various biological actions of tumor-derived MVs and their potential role in tumor biology.

**Key Words:** Microvesicles; exosomes; angiogenesis; tumor niche; stem cells; cancer microenvironment.

**INTRODUCTION**

The cellular composition of tumors is heterogeneous and consists of cells that interact with each other in a well-orchestrated process aimed to create the most favorable developmental conditions [1]. Two distinct compartments are present in tumors, namely: the parenchyma, composed of the actual tumor cells, and the stroma, comprising a combination of non-malignant cells along with elements of the connective tissue including blood and lymphatic vessels, fibroblasts and inflammatory cells [2]. These cellular components strictly interact and communicate with each other. In the stroma extracellular macromolecules are also present, such as collagen, fibronectin, fibrin, proteoglycans and hyaluronan in different proportions according to tumor type; these molecules provide a scaffold for tumor cells. All tumors require stroma, in varying amounts, which guarantees nourishment, as well as elimination of waste products and provision of structural organization; the amount of stroma is not correlated to the level of malignancy of the tumor, and is not only present in solid tumors but also in hematopoietic tumors. In recent years, the stroma has been considered a very important component of tumors, critical for both tumor maintenance and growth. The stromal microenvironment is able to actively alter tumor cell behavior, favoring tumor cell proliferation and genetic alterations that may lead to tumor progression and metastatic diffusion [2]. The cooperation of different cell types to create a positive environment for cancer development requires appropriate cell-to-cell communication. Cells are able to communicate with each other by means of soluble molecules such as cytokines and chemokines [3]. In addition to soluble factors, communication between cells may involve direct cell-to-cell contact via cytonemes that connect neighboring cells, allowing transfer of surface-associated molecules, and tunneling nanotubules which not only enable the transfer of surface molecules, but also of cytoplasmic components [4,5]. Moreover, cells may exchange information through the release of exosomes/microvesicles, able to transfer proteins, receptors, bioactive lipids, mRNA and microRNA from the cell of origin to recipient cell that may modify their phenotype and function [6]. Microvesicles released from different cell types have been recognized as being an integral component of the complex network of mediators involved in the exchange of biological information among cells within the tumor microenvironment [1].

Vesicles released from cells, both *in vitro* and *in vivo,* are a mix of exosomes-derived from the endosomal membrane compartment- and shedding vesicles, originated by direct budding from the cell plasma membrane [7-9]. Therefore, in this review we will collectively call them microvesicles (MVs). Once shed from the cell of origin, MVs may remain in the surrounding extracellular space or may enter biological fluids facilitating a long-distance transfer of information. In physiological conditions, most of the MVs present in the circulation are released from platelets [10], with a smaller amount from other blood cells and endothelial cells [11]. Tumor cells are an important source of MVs, and tumor-derived MVs are detectable within biological fluids in cancer patients and correlate with a poor prognosis [12, 13].

The MVs released from tumor cells may transfer molecules to stroma cells that may be potentially involved in the stimulation of angiogenesis and in the remodeling of extra cellular matrix [1]. In addition, tumor-derived MVs may

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favor tumor cell escape from immune surveillance, mediate resistance to chemotherapeutic drugs and favor metastatic niche formation [13-16].

**BIOGENESIS OF MVs**

MVs are thought to form via two distinct mechanisms [8, 9]. The first mechanism implies blebbing of plasma membranes with subsequent production of shedding vesicles, also defined as ectosomes or microparticles. The second mechanism involves the endosomal processing and release of cytosol-containing plasma membrane material, as exosomes. Apoptotic bodies released from dying cells are another type of shedding vesicle, often containing DNA as remnants of nuclei.

Shedding vesicles are a heterogeneous population of vesicles with sizes ranging from 100 nm to 1000 nm, originating from an extrusion of the cell membrane in a process involving calcium influx, cytoskeleton reorganization and redistribution of membrane components and formation of membrane nanodomains [17]. Shedding vesicle membranes contain high levels of cholesterol and other molecules commonly associated with lipid rafts, including tissue factor (TF) and flotillin-1 [18] and present an abundance of phosphatydilserine (PS) and other markers [9]. Formation of shedding MVs follows the exposure of PS residues on the outer surface of cells leading to budding of plasma membranes that collect specific transmembrane and cytosolic proteins [9] (Figure 1). Blebbing of plasma membranes is associated with changes in membrane lipid asymmetry. This process is controlled by several enzymes such as calpain, flippase, floppase, scramblase and gelsolin [19]. The increase of cytosolic $Ca^{2+}$ following cell stimulation leads to alteration of the transmembrane enzymatic balance resulting in surface exposure of PS (Figure 1). The $Ca^{2+}$-dependent proteolysis of the cytoskeleton results in the induction of shedding of MVs from the cell surface [20]. Therefore, all stimuli that increase intracellular $Ca^{2+}$ levels, such as bacterial lipopolysaccharides, inflammatory cytokines (tumor necrosis factor-α, interleukin 1 *etc*), aggregated low-density lipoproteins, or reactive oxygen species may stimulate MV release. In glial cells, acidic sphingomyelinase (aSMase) and the P2X7 receptor are considered to be involved in MV release by an ATP-dependent mechanism (dying cells release ATP) and activation of P2X7 receptor leading to aSMase activation with rapid sphingomyelin hydrolysis [21]. Sphingomyelin hydrolysis results in increased membrane fluidity leading to membrane blebbing and MV shedding. Dying cells within the tumor, by releasing ATP, may stimulate other cells to generate pro-inflammatory cytokine-containing MVs.

Shedding MVs may carry specific molecules of the cell of origin, for instance MVs derived from gliomas carry oncogenic growth factor receptors (*e.g.,* EGFRvIII) [22]. Moreover, shedding MVs may also contain cytokines and chemokines (e.g., IL-1β, and IL-8), VEGF and fibroblast growth factor 2 derived from the cell of origin [2].

Exosomes are a more homogeneous population of vesicles that are smaller in size (from 30 nm up to 120 nm), but the biogenesis of exosomes is mostly unknown. A sorting signal that is common for all cell types has not been identified

[23], and in different cell types the exocytosis of exosomes is regulated by distinct cellular pathways [24, 25]. The constitutive release of exosomes is thought to be triggered by a $Ca^{2+}$-independent mechanism, whereas the consequent release following cell stimulation is regulated by $Ca^{2+}$ . Exosomes originate from the endosomal compartment. After formation of early intracellular endosomes following inward invaginations of the cell membrane, under the control of the endosomal sorting complex required for transport (ESCRT), there is an evolution towards late endosomes/multivesicular bodies which may be released from the cell following fusion with the cell membrane [26, 27]. Although there is no clear evidence of ESCRT involvement in the molecular composition of exosomes, some ESCRT components such as Alix, known to be required for sorting of the transferrin receptor [28], are detectable within exosomes [29]. It has been also demonstrated the involvement of small GTPases RAB27a and RAB27b in the release of exosomes by human tumor cells [30].

Other studies indicate the involvement of ceramide that promotes membrane invaginations in the mechanism of exosome release [31]. In comparison with shedding MVs, exosomes contain a different set of molecules such as Alix, TSG101, HSC70, CD63, CD81, and CD9.

Moreover, the lipid composition of exosomes, having a low content of PS, differs from that of shedding MVs [32], whilst nucleic acids are present in both MV types. The biological fluids, along with the MVs released *in vitro* by cells, contain a mixture of exosomes and shedding vesicles, and to date, functional differences between exosomes and microvesicles have not been comprehensively investigated.


**MVs AS MEDIATORS OF INTERCELLULAR COMMUNICATION**

Cell-derived MVs recognize target cells by means of surface molecules that enable specific interactions. Some of these molecules are common to all shedding vesicles, whilst others are more selective. For example, tumor- and neutrophil-derived MVs present an abundance of metalloproteinases and proteolytic enzymes known to be instrumental in inflammation and tumor progression. Platelet-derived MVs are enriched with integrins and P-selectin, important for coagulation, and macrophage-derived vesicles express the P-selectin glycoprotein ligand-1, instrumental for macrophage-binding to platelets [8]. Therefore, the molecular composition of MVs may be different according to the diverse cell types involved, and specific for differential enrichment processes [8]. Recipient cell behavior may change after interaction with MVs, as a consequence of direct receptor-mediated stimulation, or due to transfer of proteins, receptors, bioactive lipids or nucleic acids. Indeed, MVs may induce direct cell stimulation by surface interaction, as in the case of MVs originating from platelets, monocytes or tumor cells that express the tissue factor on their membrane. These particular MVs interact with P-selectin expressed by macrophages, polymorphonuclear neutrophils and platelets. Molecules of the potent procoagulant anionic aminophospholipid PS present on the surface of MVs are conducive for the assemblage of coagulation factors, with consequent activation of the coagulation cascade reaction. Through a

surface-mediated interaction, MVs released from platelets may directly activate endothelial [33], inflammatory [34,35] and malignant human hematopoietic cells [6].

An additional role for MVs is the transfer of receptors and delivery of proteins to target cells. When MVs fuse with recipient cells they may transfer receptors and ligands. This mechanism has been demonstrated with regard to the adhesion molecule CD41, which can be transferred, via MVs, from platelets to endothelial cells [36] or tumor cells [37] as well as for the Fas-ligand and for chemokine receptors. Transfer of the Fas-ligand from tumor cells to activated T cells may promote T cell apoptosis and favor tumor escape from immune surveillance [38]. Transfer of the CXCR4 and CCR5 chemokine receptors may favor the entry of the HIV1 virus into non-lympho-hematopoietic cells, by acting as co-receptors [39, 40].

Moreover, MV-mediated transfer of biologically active proteins may modify the function of the recipient cells. For example, MV-encapsulated caspase-1, released from endotoxin-activated monocytes, was shown to induce apoptosis of vascular smooth muscle cells [41]. In addition, tumor-derived MVs may transfer oncogene products to adjacent cells leading to a subsequent change in their phenotype [22].

As MVs contain selected patterns of mRNA and microRNA (miRNA), they may transfer genetic information between cells. These particular nucleic acids are associated with ribonucleoproteins involved in the intracellular traffic of RNA [42]. The concentration of selected RNA species in MVs suggests a regulated process of RNA compartmentalization [42]. Ratajczak *et al.* [43] demonstrated that MVs derived from murine embryonic stem cells are able to induce epigenetic reprogramming of adult hematopoietic stem/progenitor cells by a horizontal transfer of mRNA. In addition, Valadi *et al.* [44] demonstrated the exosome-mediated transfer of mRNAs between mast cells, and their subsequent translation into proteins. We also found that MVs derived from human endothelial progenitor cells (EPC) may activate an angiogenic program in recipient quiescent endothelial cells by transferring selected patterns of mRNA [45]. The MV-mediated delivery of human mRNA to mouse cells, resulting in protein translation, has been shown *in vivo* as well [46, 47]. More recently, Aliotta *et al.* [48] demonstrated that bone marrow cells may undergo tissue-specific changes in mRNA, either by MV-mediated direct delivery of mRNA, or by induction of tissue specific mRNA. The latter phenomenon was related to long term stable change in genetic phenotype of recipient cells related to miRNA shuttled by MVs. The MV mediated changes in cell phenotype was also observed for brain, hearth and liver cells, suggesting that this is a general phenomenon [48]. The functional changes induced by MVs in recipient cells also depend on the transfer of miRNA present in lung derived MVs [42, 44, 49]; for example Yuan *et al.* [49] demonstrated that MVs can transfer a subset of miRNA to mouse embryonic fibroblasts *in vitro*. We have demonstrated that miRNAs are selectively accumulated within MVs released by adult human mesenchymal stem cells [42], and the gene ontology analysis of predicted and validated targets suggests that the highly expressed miRNA may be involved in multi-organ development, cell survival, differentiation and immune system regulation. Moreover, these miRNAs were also found to

be functional in recipient cells suggesting that the MV-mediated transfer of miRNA-regulators of protein translation-can alter the expression of gene products in adjacent cells.

## TUMOR-DERIVED MVs ARE AN INTEGRAL COMPONENT OF THE CANCER MICROENVIRONMENT

Due to their pleiotropic effects, tumor-derived MVs may profoundly influence the cancer microenvironment by interacting with stroma cells and favoring tumor cell escape from immune surveillance or promoting resistance to chemotherapeutic drugs [1]. Moreover, tumor-derived MVs, by entering biological fluids, may act at distant sites from the tumor, facilitating blood coagulation and/or metastatic niche formation [14-16].

MV release from tumor cells is regulated by a small GTP-binding protein- ARF6- whose inhibition is associated with PKC-mediated phosphorylation of myosin light-chain with the consequent block of MV shedding [50]. In addition, MVs modulate expression of tumor cell components involved in cell adhesion and motility [51]. Moreover, tumor-derived MVs express TF that has a central role in triggering the coagulation cascade, and a correlation between the presence of TF-bearing MVs and an increased risk of thromboembolic events has been established in cancer patients [51]. Tumor MVs also overexpress PS that provides a catalytic site for the coagulation cascade [52, 53]. MV-triggered coagulation not only has systemic implications, but also contributes to alteration of the tumor microenvironment, leading to proliferation of dormant tumor stem cells [25, 54] or activation of the angiogenic shift [53].

Table 1 summarizes the content and functions of extracellular vesicles released from different types of tumor cells. Tumor-derived MVs may facilitate tumor invasion and metastases as they carry EMMPRIN a transmembrane glycoprotein- identified as a tumor-derived factor that can stimulate matrix metalloproteinase expression in fibroblasts [55]. Therefore, degradation of extracellular matrix by fibroblasts may be under tumor cell control by the microvesicular release of EMMPRIN.

Castellana *et al.* [15, 53] suggested a bidirectional communication between tumor and normal stroma cells through mutual shedding of MVs, with a resulting possible increase in tumorigenicity. Tumor-derived MVs activate fibroblasts and promote MV shedding. In turn, MVs derived from fibroblasts increase migration and invasion of highly metastatic prostate carcinoma cells. This mechanism is, at least in part, dependent on membrane-bound CX3CL1/fractalkine ligand for chemokine receptor- CX3CR1 [15]. Lung cancer-derived MVs are also able to recruit and activate stromal fibroblasts and endothelial cells, thus creating an environment favorable to tumor growth [56]. In addition to delivering receptors, tumor-derived MVs may carry biological active proteins, for example, Baj-Krzyworzeka *et al*. [57] demonstrated that tumor-derived MVs contain interleukin-8 and modulate chemokine production by human monocytes, thus explaining the observation that MVs are able to mimic the effect of tumor cells in monocytes [58]. Tumor MVs may also directly contribute to extracellular matrix degradation, as they may express matrix metalloproteinases (MMP)

and extracellular MMP inducer at their surface, evident in MVs that are released from prostate carcinoma cells [15]. Secretion of hsp90α from tumor cells via MVs may also promote cancer cell invasion by activating plasmin [59].

Another class of proteins that can be delivered by tumor-derived MVs to neighboring cells is the products of oncogenes. Al Nedawi *et al.* [22] showed the transfer by MVs of the oncogenic form of the epidermal growth factor receptor EGFRvIII, exclusively expressed in a subset of aggressive gliomas, to non-aggressive tumor cells. The horizontal propagation of this oncogene product results in the activation of signaling pathways involved in cell survival and cell growth, including mitogen-activated protein kinase and Akt, and changes the expression of EGFRvIII-regulated genes such as vascular endothelial growth factor, P27 and Bcl-$X_L$. As a consequence, recipient cells undergo morphological transformation and acquire anchorage-independent growth properties [22]. More recently, Antonyak *et al.* [60] demonstrated that MVs derived from breast carcinoma and glioma cells induce transformation by transferring tissue transglutaminase and fibronectin to fibroblast and epithelial cells.

Tumor-derived MVs may also modulate angiogenesis within the cancer microenvironment. Neo-angiogenesis is critical for solid tumor growth and invasion, as the vasculature provides nutrients, oxygen and access to the circulation. Recent studies demonstrate that tumor endothelial cells possess a distinct phenotype, differing from normal endothelial cells at a molecular and functional level. Mechanisms leading to this altered phenotype are still a matter of debate, but several lines of evidence indicate a leading role of tumor MVs in driving these alterations [61].

MVs shed by ovarian cancer cells expressing CD147/extracellular MMP inducer promote an angiogenic phenotype in endothelial cells *in vitro* [62]. Silencing CD147 by small interfering RNA in ovarian cancer cells suppresses the angiogenic potential of MVs, suggesting that the proangiogenic activity depends on a CD147-mediated mechanism.

It has been also shown that the expression of sphingomyelin by tumor-derived MVs contributes to the stimulation of endothelial cell migration, invasion, and tube formation *in vitro*, and the induction of *in vivo* neovascularization [63]. Another constitutive component of exosomes, tetraspanin, is enriched in tumor MVs and may contribute to the induction of endothelial cell activation and angiogenesis [64]. Al-Nedawi *et al.* [65] suggested that MV-mediated transfer of oncogenic EGFR from tumor to endothelial cells activates autocrine expression of VEGF, leading to angiogenic activation. Activated endothelial cells in turn may transfer the Notch ligand Delta-like 4 (DlI4) *via* MVs to quiescent endothelial cells, thus propagating the angiogenic signal within the tumor microenvironment [66]. Moreover, MVs, by delivering metalloproteinases, may induce stromal remodeling leading to endothelial [67] and tumor cell invasion [68].

It has been suggested that MVs may enhance the metastatic potential of tumor cells, either by acting on the tumor microenvironment itself, in order to favor the entry of cells into the circulation, or by creating the so-called "pre-metastatic niche", a prerequisite for tumor cell implantation. Indeed, rat pancreatic adenocarcinoma-derived MVs

modify the lung microenvironment allowing the development of lung metastases [69]. The authors of this study suggest that *in vivo* pre-metastatic changes result from cooperation of both exosomes and soluble matrix components.

Another mechanism involved in MV-induced changes in the cancer microenvironment is the reprogramming of normal stromal and endothelial cells by the transfer of genetic material from cancer cells. Epigenetic changes induced by MVs depend on the delivery of specific subsets of mRNA and miRNAs. Baj-Krzyworzeka *et al.* [70] showed that tumor-derived MVs may transfer tumor mRNA to bystander cells, such as monocytes, and Ratajczak *et al.* [43] demonstrated that transfer of mRNA by MVs may reprogram hematopoietic progenitors. Skog *et al.* [71] showed that MVs released from glioblastoma contain a variety of mRNA and microRNA transcripts related to cell migration, angiogenesis and proliferation. This particular study showed that mRNA for a reporter protein is translated in the recipient cells, and confirmed that MVs released by tumor cells may deliver specific and functional RNAs. MVs shed by colorectal tumor cells are enriched with cell cycle-related mRNAs and promote endothelial cell proliferation [72]. By a mechanism of horizontal transfer of mRNA, normal quiescent endothelial cells can be also activated by MVs released from the EPC [45] which have been also implicated in the tumor angiogenic shift [61]. MVs derived from lung and prostate carcinomas were shown to induce phenotypic changes in marrow cells [73, 74]. More recently, Balaj *et al.* [75] provided evidence that tumor-derived MVs contain retro-transposon elements and amplified oncogene sequences, thus expanding the range of genetic information transferred by tumor MVs to stromal and endothelial cells, facilitating a receptible microenvironment for cancer development. Guescini *et al.* [76] demonstrated that exosomes released from astrocytes and glioblastoma cells carry also mitochondrial DNA. Recently, it has been shown that exosomes released by breast cancer cells induce a myofibroblast phenotype and function in adipose tissue-derived mesenchymal stem cells via transforming growth factor-β/small mother against decapentaplegic (SMAD)-mediated signaling pathway [77]. This interesting observation suggests that tumor derived exosomes can convert mesenchymal stem cells into tumor promoting myofibroblasts. All cell types may contribute to MV shedding within the tumor mass, which contains a heterogeneous population of cells with different proliferation and differentiation potentials. However, we recently found that in renal cancer, the MVs that retained angiogenic properties were specifically those derived from tumor stem cells [16]. These MVs, in contrast with those derived from more differentiated tumor cells, contained pro-angiogenic mRNAs and miRNAs that may be involved in tumor progression and metastases. MVs released from renal cancer stem cells not only may contribute *in vivo* to trigger local angiogenesis, but may also coordinate metastatic diffusion by creating a favorable environment in the lung for tumor cell implantation. In this study, we proposed that the RNA content of MVs plays a critical role as RNase treatment of MVs significantly inhibited the *in vitro* and in particular the *in vivo* biological effects of MVs. Recently, Zhang *et al.* [78] showed that tumor stem cells not only initiate tumors but may promote metastases in virtue of their peculiar content of tumorigenic miRNAs. Tumor stem cell-derived MVs may be instrumental in transferring RNAs to neighboring cells, inducing changes in their phenotype that may sustain an

unfavorable outcome of the tumor.

**ROLE OF TUMOR MVs IN CHEMORESISTANCE AND IMMUNE ESCAPE**

Another method by which MVs may facilitate the tumor cell survival is their involvement in multi-drug resistance. It has been suggested that MVs may actually expel chemotherapeutic agents from tumor cells resistant to chemotherapy [79]. Indeed, chemotherapy-resistant tumor cells release significantly more MVs loaded with chemotherapeutic drugs than those that are chemotherapy-sensitive. Through this mechanism tumor cells are able to reduce the intracellular concentration of the drug in order to protect themselves from dying.

Tumor MVs have been also extensively studied for their ability to modulate the immune response [24, 80]. Human tumors are able to develop strategies for facilitating protection from the host immune system. The adopted mechanisms, are highly varied, even between tumors that share the same histology [81]. The genetic instability of tumors is responsible for tumor heterogeneity and for the antigenic epitope profile changes of tumor cells [82].

The progression of a malignancy is the result of failure both in non-immune and immune surveillance processes, leading to the selection of a population of cancer cells able to resist the attack of the immune system as they are either less immunogenic or more resistant to apoptosis. This process, known as "cancer immuno-editing", leads to the survival and uncontrolled proliferation of immune resistant cancer cells [83]. Several studies have addressed the role of MVs in this context. MVs may either stimulate anti-tumor immune responses or contribute to create favorable conditions for tumor immune escape depending on the cell of origin and their molecular composition.

MVs derived from mature dendritic cells have been proposed as vaccines to stimulate the efficient antitumor cytotoxic T-lymphocyte response [84]. Indeed, MVs may stimulate immune responses by direct peptide-MHC complex presentation to T cells; transfer of antigens or peptide-MHC complexes to dendritic cells; or activation of natural killer cells and macrophages. In addition, MVs expressing CD40L may stimulate B cell activation and antibody production [85]. Conversely, increasing lines of evidence indicate that tumor-derived MVs possess immune-suppressive properties. Tumor-derived MVs may protect tumor cells from inflammatory and immune responses by inducing T cell apoptosis, impairing monocyte differentiation into dendritic cells and inhibiting cytotoxic responses [86]. Valenti *et al.* [87] demonstrated that MVs released by human melanoma and colorectal carcinoma cells may tune monocytes into transforming growth factor-β-producing myeloid cells, with suppressive activity on T lymphocytes. More recently, Lima *et al.* [88] suggested that the down-regulation of the host inflammatory and immune response may depend on PS exposure on tumor-derived MV membranes that favors the establishment of melanoma metastases. In addition, tumor-derived MVs bearing the pro-apoptotic molecule Fas-ligand can induce death of activated T cells [89]. Further evidence of immune suppression by MVs was demonstrated in that exosomes capable of inducing T cell apoptosis were isolated from ascites and serum of ovarian cancer patients [90, 91]. MVs derived from human colorectal cancer cells were also

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shown to induce T-cell apoptosis by a mechanism dependent on Fas-ligand and TRAIL [92, 93]. Yang *et al.* [94] recently demonstrated that tumor-derived exosomes may induce tumor antigen specific immunosuppression by modulation of antigen presenting cell functions. In addition, cancer exosomes were shown to suppress T cell activity through adenosine production triggered by CD39 and CD73 expressed on the exosome surface [95] and to inhibit natural killer cell cytotoxicity via the human NKG2D ligand MICA*008 [96]. Another way by which MVs may protect tumor cells is the vesicular shedding of terminal components of complement from the cell plasma membrane allowing escape from antibody-mediated immune responses [97].

## CONCLUSION

It has become increasingly clear that exosomes/MVs released from tumor cells are not only able to modify the tumor microenvironment, but can also act further afield by entering the circulation and other biological fluids. Therefore, MVs may be regarded as "messengers" that deliver multiple messages and complex information. Due to their pleiotropic biological actions, MVs are now considered an integral component of the complex network of cell interactions involved in tumor growth and diffusion. Tumor-derived MVs may influence the phenotype of stromal cells by transferring biologically active molecules and gene products. In particular, the exchange of genetic information mediated by MVs allows a cross-talk between tumor and stromal cells to establish a favorable tumor niche, and to promote tumor growth, invasiveness and progression. The horizontal transfer of gene products, considered to be impossible until now, emerges as a potential mechanism of epigenetic alterations in stromal cells with consequent reprogramming of their phenotype and functions. Moreover, tumor-derived MVs possess immune-suppressive properties that may contribute to tumor escape from the immune response. The understanding of the biological action of tumor-derived MVs, and the identification of signals that they deliver is fundamental for designing new therapeutic strategies aimed at interfering with the tumor microenvironment.

## CONFLICT OF INTEREST

Ciro Tetta is a full time employee of Fresenius Medical Care. All the other authors declared no competing interests.

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**Legend of Figure 1**

Schematic representation of the biogenesis of shedding vesicles and exosomes.

Shedding vesicles originate from blebbing of the cell membrane, associated with changes in membrane lipid asymmetry. The increased calcium concentration following cellular activation leads to: i. inactivation of the ATP-dependent aminophospholipid translocase (flippase) with consequent PS translocation to the external leaflet of the cell membrane bilayer; ii.inactivation of floppase, an ATP-dependent enzyme that catalyzes lipid transfer from the inner leaflet of the cell membrane to the outer leaflet, probably in conjunction with flippase; iii. activation of scramblase that catalyzes bidirectional movement of phospholipids back and forth between the two leaflets of the cell membrane with collapse of the cell membrane phospholipid asymmetry; vi. activation of calpain that cleaves the long actin filaments of the cytoskeleton, and activation of gelsolin in platelets, that cleaves the actin-capping proteins, allowing a reorganization and disruption of the cytoskeleton, leading to membrane budding.

Exosomes originate from the endosomal compartment. From intracellular early endosomes they evolve into multivesicular bodies, probably under the control of the endosomal sorting complex required for transport (ESCRT) and they may then be released into the extracellular space after fusion with the cell membrane.

**Table 1. Functions of tumor derived vesicles**

| Tumor type | Type of vesicles | Content | Function | Ref. |
|---|---|---|---|---|
| Lung carcinoma | Microvesicles | EMMPRIN | Tumour stroma interaction | 55 |
| Lung carcinoma | Microvesicles | None | Angiogenesis and metastasis | 56 |
| Lung carcinoma | Microvesicles | Lung specific RNAs | Phenotypic changes in marrow cells | 73 |
| Pancreatic adenocarcinoma, colorectal adenocarcinoma, lung carcinoma. | Microvesicles | mRNA for VEGF, HGF, IL-8 and surface determinants (CD44H) | Activation of tumour infiltrating monocytes | 57 |
| Prostate carcinoma | Microvesicles | Matrix metalloproteinases; Exchange of receptors (CX3CL1/fractalkine-CX3CR1) | Establishment of a favorable tumor niche | 15 |
| Prostate carcinoma | Microvesicles | Prostate specific RNAs | Prostate specific gene expression in human bone marrow cells. | 74 |
| Breast cancer | Exosomes | Hsp90alpha | Increase in cancer cell motility | 59 |
| Gliomas | Microvesicles | Oncogenic form of EGFRvIII | Tumour progression | 22 |
| Breast carcinoma and glioma cells | Microvesicles | Trans glutaminase, fibronectin | Transformation | 60 |
| Ovarian cancer | Microvesicles | CD147/extracellular matrix metalloproteinase inducer | Angiogenesis | 62 |
| Human squamous carcinoma, alveolar basal epithelial adenocarcinoma and colon cancer | Microvesicles | Oncogenic EGFR | Angiogenesis by induction of autocrine VEGF production | 65 |
| Rat pancreatic adenocarcinoma | Exosomes | CD44v6 | Lung metastasis | 69 |
| Human fibrosarcoma and prostate carcinoma | Microvesicles | Sphingomyelin | Angiogenesis | 63 |
| Glioblastoma | Microvesicles | mRNA, microRNA, proteins | Tumor growth and diagnostic bio markers | 71 |
| Colorectal carcinoma | Microvesicles | Cell cycle related mRNA | Angiogenesis | 72 |
| Glioblastoma, medulloblastoma, atypical teratoid rabdoid tumor and melanoma | Microvesicles | Retro-transposon elements, amplified oncogene sequences- | Tumor growth and progression | 75 |
| Renal cancer stem cells | Microvesicles | mRNA and microRNA | Angiogenesis, tumor invasion and metastasis | 16 |
| Glioblastoma | Exosomes | Mitochondrial DNA | Tumor progression | 76 |
| Breast cancer | Exosomes | None | Conversion of MSCs in tumor associated myofibroblasts | 77 |

**Abbreviations:** EMMPRIN, extracellular matrix metalloproteinase inducer also known as basigin and CD147; VEGF, vascular endothelial growth factor; HGF, hepatocyte growth factor; IL-8, interleukin-8; EGFR, epidermal growth factor receptor; MSCs, mesenchymal stem cells.